JOURNAL OF CLINICAL MICROBIOLOGY, June 1976, p. 643-645
Vol. 3, No. 6

Printed in U.S.A.

Effect of Volume of Blood Cultured on Detection of Bacteremia

M. MARSHA HALL, DUANE M. ILSTRUP, AND JOHN A. WASHINGTON II*
Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55901

Received for publication 23 February 1976

The rates of recovery of bacteria from vented vacuum blood culture bottles containing 50 and 100 ml of a soybean-casein digest broth were compared. Overall, more isolates were recovered from the larger bottle; moreover, gram-negative bacilli and especially *Pseudomonas aeruginosa* were recovered significantly more frequently ($P < 0.01$) from the 100-ml bottle.

Although it has recently been recommended that blood for culture be inoculated on a 5 to 10% (vol/vol) basis into two evacuated screw-capped bottles containing 50 to 100 ml of medium (2), Bartlett's survey of contemporary blood culture practices showed that the majority of clinical microbiology laboratories collected 10 ml of blood, which was equally divided and inoculated into two culture bottles containing 50 ml of broth (1). Nonetheless, the manufacturers of blood culture bottles have provided their customers with the choice of purchasing 50- or 100-ml bottles.

Since 1968, we have routinely inoculated (on a 10% [vol/vol] basis) at least two 100-ml vacuum blood culture bottles on the assumption that culture of a large sample of blood would enhance the recovery of small numbers of bacteria. A dicision to use these larger bottles is of considerable consequence to most laboratories because of their greater expense and because of the larger amount of incubation space they require. Since we were unaware of any data demonstrating the importance of the volume of blood cultured and, consequently, of the volume of medium inoculated, we decided to examine this issue by comparing bacterial recovery rates from 50- and 100-ml vacuum blood culture bottles inoculated in parallel with blood from patients with suspected bacteremia.

A sufficient volume of blood was collected with a sterile needle and syringe, after skin preparation with povidone-iodine, by members of a phlebotomy team to inoculate (on a 10% [vol/vol] basis) two blood culture bottles containing 100 ml of a soybean-casein digest broth (tryptic soy [TSB], Difco Laboratories) and one bottle containing 50 ml of the same medium. All media contained 0.025% sodium polyanetholsulfonate and were packaged under vacuum with CO_2. Upon their receipt in the laboratory, one of the 100-ml bottles was transiently vented with a sterile cotton-plugged needle; the other 100-ml bottle remained unvented during its period of observation. We elected to vent the 50-ml bottle and to compare its performance with that of the vented 100-ml bottle because, in a previously reported study (8) comparing vented and unvented vacuum blood culture bottles with TSB, we had detected *Pseudomonas* and *Candida* significantly more frequently and more rapidly in the vented bottle and had found no other significant differences between the two. All bottles were incubated at 35 C; they were inspected daily for 7 days and again after 13 days of incubation.

Routine subcultures of macroscopically negative bottles were performed on the day of their collection and 48 h later, as described elsewhere (8). Methods of statistical analysis were based on those described by Cochran (5).

Exclusive of presumed contaminants, a total of 311 isolates (Table 1) was recovered from 145 patients. There were 216 isolates recovered from both the 50- and 100-ml bottles, 31 from the 50-ml bottle only, and 64 from the 100-ml bottle only. Gram-negative bacilli, especially *Pseudomonas aeruginosa*, were recovered significantly more frequently ($P < 0.01$) from the 100-ml than from the 50-ml bottle. Significant differences between the two bottles in isolation rates of gram-positive cocci and of yeasts were not observed.

In examining the data from patients with two or more positive sets of blood cultures, there were 11 patients whose positive cultures were limited to the larger bottle; the converse did not occur. Overall, in these patients with multiple sets of positive cultures there were 137 and 105 isolates from the 100- and 50-ml bottles, respectively.

The volume of blood to be cultured has not been examined critically in recent years. Fundamental differences among detection methods,

TABLE 1. *Numbers of isolates[a] in blood culture bottles containing 100 and 50 ml of TSB*

Isolate	Isolates (no.) in: 100-ml bottle only	50-ml bottle only	Both 100- and 50-ml bottles	*P* value
Gram-negative bacilli				
Enterobacteriaceae	23	10	74	
Pseudomonadaceae	13	1	31	
Bacteroidaceae	3	3	12	
Other	1	0	0	
Subtotal	40	14	117	<0.01
Gram-positive cocci				
Micrococcaceae	15	9	42	
Streptococcaceae	7	6	48	
Peptococcaceae	0	0	1	
Subtotal	22	15	91	NS[b]
Yeasts				
Candida	2	2	8	NS

[a] Exclusive of single sets of cultures positive for *Bacillus, Corynebacterium, Propionibacterium,* and *Staphylococcus epidermidis* (presumed contaminants).
[b] NS, Not significant.

media, atmospheres of incubation, and subculture times and procedures have precluded analysis of the importance of this point in the many recent articles reporting the results of comparisons of methods for detection of bacteremia. In this study, the media, the additives, the atmospheres of incubation, and the timing and methods of subcultures were identical in the test systems being compared. The only differences between the systems were the inoculation of 5 ml of blood into the 50-ml bottle of TSB and the inoculation of 10 ml of blood into the 100-ml bottle of TSB.

The results demonstrated that a larger number of isolates overall and that significantly more ($P < 0.01$) gram-negative bacilli were recovered from 10 ml than from 5 ml of blood. These results substantiate those reported previously by us in a study comparing the isolation rates of bacteria from two 100-ml bottles, each inoculated with 10 ml of blood, with those from two tubes, each inoculated with 2 ml of blood (7); however, since the media in the tubes differed from those in the bottles, it was difficult for us to ascribe the significantly lower isolation rates of bacteria from the tubes exclusively to the smaller volume of blood which they received.

Because of the fact that the order of magnitude of bacteremia has generally been found to be quite low (9, 11), except in bacteremias reported in neonatal septicemia (6), one would anticipate that the isolation rates of bacteria are directly related to the volume of blood cultured. In our study, statistically significant effects of the volume of blood cultured were limited to the gram-negative bacilli for reasons that remain unexplained.

Although it might be tempting to conclude that the use of 100-ml bottles would reduce or even eliminate the necessity of obtaining multiple sets of blood cultures in 50-ml bottles, it is important to bear in mind that clinically significant bacteremias, other than those associated with endocarditis, are usually intermittent (3), and their detection appears to require the collection of several separate sets of blood cultures. In a previous study of 80 bacteremic patients from whom at least three separate sets of blood samples (10 ml into each of two 100-ml bottles) were cultured within a 24-h interval, it was found that 64 (80%) cultures were positive in the first set, 71 (89%) in the first two sets, and 79 (99%) in the first three sets (10). In patients with endocarditis, the bacteremia is usually continuous (3) and can in nearly all cases be detected in the first two sets of blood cultures (11). It is our practice, therefore, to require collection of at least two separate sets of blood cultures within a 24-h interval from each patient with suspected bacteremia. By the same token, we require prior consultation with the laboratory's staff for collection of more than three sets of blood cultures within a 24-h interval.

We recommend, therefore, that the 100-ml bottle, inoculated on a 10% (vol/vol) basis, be used for blood cultures. The use of two such bottles, one vented and one unvented, on a routine basis appears to be necessary to ensure the recovery of anaerobic, facultatively anaerobic, and aerobic bacteria, as well as yeasts from blood (4, 8). Obviously, the volume of blood collected is reduced in infants and children. Our rule of thumb is to continue to divide the blood between two bottles as long as at least 10 ml has been collected. Any lesser amount is inoculated into one bottle only, and this bottle is vented upon its receipt in the laboratory because of the rarity of anaerobic bacteremia in infants and young children.

LITERATURE CITED

1. **Bartlett, R. C.** 1970. Contemporary blood culture practices, p. 15-35. *In* A. C. Sonnenwirth (ed.), Bacteremia: laboratory and clinical aspects. Charles C Thomas Publisher, Springfield, Ill.
2. **Bartlett, R. C., P. D. Ellner, and J. A. Washington II.** 1974. Cumitech 1: blood cultures. American Society for Microbiology, Washington, D.C.
3. **Bennett, I. L., Jr., and P. B. Beeson.** 1954. Bacteremia: a consideration of some experimental and clinical aspects. Yale J. Biol. Med. 26:241-262.
4. **Blazevic, D. J., J. E. Stemper, and J. M. Matsen.** 1975. Effect of aerobic and anaerobic atmospheres on isola-

tion of organisms from blood cultures. J. Clin. Microbiol. 1:154–156.
5. **Cochran, W. G.** 1950. The comparison of percentages in matched samples. Biometrika 37:256–266.
6. **Dietzman, D. E., G. W. Fischer, and F. D. Schoenknecht.** 1974. Neonatal *Escherichia coli* septicemia—bacterial counts in blood. J. Pediatr. 85:128–130.
7. **Hall, M., E. Warren, and J. A. Washington II.** 1974. Detection of bacteremia with liquid media containing sodium polyanetholsulfonate. Appl. Microbiol. 27:187–191.
8. **Harkness, J. L., M. Hall, D. Ilstrup, and J. A. Washington II.** 1975. Effects of atmosphere of incubation and of routine subcultures on detection of bacteremia in vacuum blood culture bottles. J. Clin. Microbiol. 2:296–299.
9. **Sullivan, N. M., V. L. Sutter, W. T. Carter, H. R. Attebery, and S. M. Finegold.** 1972. Bacteremia after genitourinary tract manipulation: bacteriological aspects and evaluation of various blood culture systems. Appl. Microbiol. 23:1101–1106.
10. **Washington, J. A. II.** 1975. Blood cultures: principles and techniques. Mayo Clin. Proc. 50:91–98.
11. **Werner, A. S., C. G. Cobbs, D. Kaye, and E. W. Hook.** 1967. Studies on the bacteremia of endocarditis. J. Am. Med. Assoc. 202:199–203.